

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of claims:

1. (currently amended) A method of preparing normalized and/or subtracted cDNAs comprising the steps of:

I) preparing uncloned full-length or full-coding length cDNA tester;

II) preparing polynucleotide drivers for normalization and/or subtraction;

III) conducting normalization and/or subtraction and removing tester/driver hybrids, non-specifically hybridized polynucleotide drivers and non-hybridized polynucleotide drivers; and

IV) recovering the normalized and/or subtracted full-length or full-coding length cDNA.

2. (previously presented) The method of claim 1, wherein the cDNA tester of step I) is a reverse transcript of mRNA in the form of uncloned cDNA.

3. (previously presented) The method of claim 1 wherein said cDNA tester is single-stranded.

4. (previously presented) The method of claim 1, wherein in step III), normalization is conducted first, followed by subtraction.

5. (previously presented) The method of claim 1, wherein in step III), subtraction is conducted first, followed by normalization.

6. (previously presented) The method of claim 1, wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

7. (canceled).

8. (previously presented) The method of claim 1, wherein step III) comprises the addition of an enzyme capable of cleaving single-stranded RNA driver nonspecifically bound to single-stranded cDNA and the cleaved single-stranded RNA driver is removed.

9. (original) The method of claim 8 wherein said enzyme is single-strand-specific RNA endonuclease.

10. (original) The method of claim 8 wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

11. (original) The method of claim 8 wherein said enzyme is RNase I.

12. (previously presented) The method of claim 1, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

13. (currently amended) The method of claim 1, wherein the preparation of said full-length or full-coding length cDNA tester comprises the following steps:

- (1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;
- (2) chemically binding a tag molecule to the diol structure of the 5' CAP (⁷MeG_{PPP}N) site of mRNA forming ~~hybrids~~; hybrids;
- (3) trapping full-length or full-coding length cDNA hybrids; and
- (4) removing single-stranded mRNA by digestion with an enzyme capable of cleaving single-stranded mRNA.

14. (original) The method of claim 13 wherein said tag molecule is digoxigenin, biotin, avidin, or streptavidin.

15. (previously presented) The method of claim 1, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

16. (original) The method of claim 15, wherein said DNA driver is cDNA.

17. (previously presented) The method of claim 1, wherein said normalization driver comprises cellular mRNA from the same library, from the same tissue, or the same cDNA population as the cDNA to be normalized.

18. (previously presented) The method of claim 1, wherein said normalization driver comprises single-stranded cDNA obtained from the same library, the same tissue, or the same cDNA population as the cDNA to be normalized.

19. (previously presented) The method of claim 1, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from the cDNA to be subtracted.

20. (previously presented) The method of claim 1, wherein said subtraction driver comprises single-stranded cDNA from a library, tissue, or cDNA population differing from the cDNA to be normalized.

21. (previously presented) The method of claim 1, further comprising a step V) of preparing a complementary strand of the recovered cDNA and cloning the resulting double-stranded cDNA.

22. (currently amended) A method of preparing normalized and/or subtracted full-length or full-coding length cDNAs comprising the steps of:

- I) preparing cDNA tester not cloned in a plasmid;
- II) preparing polynucleotide drivers for normalization and/or subtraction;
- III) conducting normalization and/or subtraction and removing tester/driver hybrids, non-specifically hybridized

polynucleotide drivers and non-hybridized polynucleotide drivers; and

IV) recovering the normalized and/or subtracted full-length or full-coding length cDNA.

23. (original) The method of claim 22, wherein in step III), normalization is conducted first, followed by subtraction.

24. (original) The method of claim 22, wherein in step III), subtraction is conducted first, followed by normalization.

25. (original) The method of claim 22, wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

26. (canceled).

27. (previously presented) The method of claim 22, wherein step III) comprises the addition of an enzyme that cleaves single-stranded RNA driver nonspecifically bound to single-stranded cDNA and the cleaved single-stranded RNA driver is removed.

28. (original) The method of claim 27, wherein said enzyme is single-strand-specific RNA endonuclease.

29. (original) The method of claim 27, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA,

RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

30. (original) The method of claim 27, wherein said enzyme is RNase I.

31. (previously presented) The method of claim 22, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

32. (previously presented) The method of claim 22, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population the cDNA is to be normalized.

33. (previously presented) The method of claim 22, wherein said normalization driver comprises single-stranded cDNA obtained from the same library, the same tissue, or the same cDNA population as the cDNA to be normalized.

34. (previously presented) The method of claim 22, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from the cDNA to be subtracted.

35. (previously presented) The method of claim 22, wherein said subtraction driver comprises single-stranded cDNA from a library, tissue, or cDNA population differing from the cDNA to be normalized.

36. (previously presented) The method of claim 22, further comprising a step V) of preparing a complementary strand of the recovered full-length or full-coding length cDNA and cloning the resulting double-stranded full-length or full-coding length cDNA.

37. (currently amended) A method of preparing normalized and subtracted full-length or full-coding length cDNA comprising the steps of:

- I) preparing cDNA tester;
- II) preparing polynucleotide drivers for normalization and subtraction;
- III) conducting the normalization and subtraction as a single step by mixing together the tester and the drivers; and
- IV) removing non-specifically hybridized polynucleotide drivers; and
- V) recovering the normalized and subtracted full-length or full-coding length cDNA.

38. (original) The method of claim 37, wherein the cDNA tester is cloned or uncloned cDNA.

39. (previously presented) The method of claim 37, wherein the cDNA tester is the reverse transcript of mRNA in the form of uncloned cDNA.

40. (previously presented) The method of claim 37, wherein the cDNA tester is single-stranded.

41. (canceled).

42. (currently amended) The method of claim 37, wherein ~~step III~~~~step IV~~ comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single-stranded cDNA and the cleaved single-stranded RNA driver is removed.

43. (original) The method of claim 42, wherein said enzyme is single-strand-specific RNA endonuclease.

44. (original) The method of claim 42, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

45. (original) The method of claim 42, wherein said enzyme is RNase I.

46. (previously presented) The method of claim 37, wherein said cDNA tester is prepared by CAP-trapping 5' end of RNA.

47. (previously presented) The method of claim 37, wherein the preparation of said full-length or full-coding length cDNA tester comprises the following steps:

(1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;

- (2) chemically binding a tag molecule to the diol structure of the 5' CAP (^{7Me}G_{PPP}N) site of mRNA forming hybrids;
- (3) trapping full-length or full-coding length cDNA hybrids; and
- (4) removing single-stranded mRNA by digestion with an enzyme that cleaves single-stranded mRNA.

48. (original) The method of claim 47, wherein said tag molecule is digoxigenin, biotin, avidin, or streptavidin.

49. (previously presented) The method of claim 37, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

50. (previously presented) The method of claim 49, wherein said DNA driver is cDNA.

51. (previously presented) The method of claim 37, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as the cDNA to be normalized.

52. (previously presented) The method of claim 37, wherein said normalization driver comprises single-stranded cDNA obtained from the same library, the same tissue, or the same cDNA population as the cDNA to be normalized.

53. (previously presented) The method of claim 37, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from the cDNA to be subtracted.

54. (previously presented) The method of claim 37, wherein said subtraction driver comprises single-stranded cDNA from a library, tissue, or cDNA population differing from the cDNA to be normalized.

55. (currently amended) The method of claim 37, further comprising a ~~step V) step VI)~~ of preparing a complementary strand of the recovered full-length or full-coding length cDNA and cloning the resulting double-stranded full-length or full-coding length cDNA.

56. (previously presented) A method of preparing normalized and/or subtracted full-length or full-coding length cDNA comprising the steps of:

- (a) preparing cDNA tester;
- (b) preparing normalization and/or subtraction RNA driver;
- (c) conducting normalization and/or subtraction in two steps in any order, or conducting normalization/subtraction as a single step and mixing the normalization/subtraction RNA driver with said cDNA tester;
- (d) adding an enzyme that cleaves single-stranded sites on RNA drivers non-specifically bound to cDNA tester;

(e) removing said single-stranded RNA driver cleaved in step
d) from the tester and removing tester/driver hybrids; and
(f) recovering the normalized and/or subtracted full-length
or full-coding length cDNA.

57. (original) The method of claim 56, wherein the cDNA tester is cloned or uncloned cDNA.

58. (previously presented) The method of claim 56, wherein the cDNA tester is a reverse transcript of mRNA in the form of uncloned cDNA.

59. (previously presented) The method of claim 56, wherein said cDNA tester is single-stranded.

60. (previously presented) The method of claim 56, wherein in step c), normalization is conducted first, followed by subtraction.

61. (previously presented) The method of claim 56, wherein in step c), subtraction is conducted first, followed by normalization.

62. (previously presented) The method of claim 56, wherein in step c), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

63. (previously presented) The method of claim 56, wherein said normalized and/or subtracted cDNA is full-length or full-coding length cDNA.

64. (previously presented) The method of claim 56, wherein the enzyme of said step d) is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

65. (previously presented) The method of claim 56, wherein the enzyme of said step d) is RNase I.

66. (previously presented) The method of claim 56, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

67. (previously presented) The method of claim 56, further comprising the step g) of preparing a complementary strand of the recovered cDNA and cloning the resulting double-stranded cDNA.

68. (previously presented) The method of claim 1, wherein said tester/driver hybrids are bound to tag molecules.

69. (original) The method of claim 68, wherein said tag molecule is avidin, streptavidin, biotin, digoxigenin, an antibody, or an antigen.

70. (previously presented) The method of claim 1, wherein said tester/driver hybrids are removed through the use of a matrix.

71. (original) The method of claim 70, wherein said matrix is comprised of magnetic beads or agarose beads.

72. (previously presented) The method of claim 71, wherein said magnetic beads or agarose beads are covered by or bound to a tag molecule that binds to a tag molecule bound to a tester/driver hybrid.

73. (previously presented) The method of claim 71, wherein said magnetic beads or agarose beads are covered by or bound to a tag molecule that binds to avidin, streptavidin, biotin, digoxigenin, an antibody, or an antigen bound to a tester/driver hybrid.

74. (previously presented) The method of claim 72, wherein said tag molecule covering said beads or bound to said beads is an antiantigen antibody, antibiotin antibody, antiavidin antibody, antistreptavidin antibody, or antidigoxigenin antibody.

75. (previously presented) The method of claim 1, wherein said tester/driver hybrid is removed using streptavidin/phenol.

76. (previously presented) The method of claim 1, wherein hydroxyapatite and nonlabeled RNA are employed to remove said tester/driver hybrid.

77. (currently amended) A method of removing RNA nonspecifically bound to DNA comprising processing nonspecifically bound RNA/DNA

hybrids having single-stranded RNA sites with an enzyme that degrades single-stranded RNA.

78. (currently amended) The method of claim 77, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or ~~comprises~~—a mixture thereof.

79. (original) The method of claim 77, wherein said enzyme is RNase I.

80. (previously presented) The method of claim 77, wherein said RNA/DNA hybrid is a product of normalization.

81. (previously presented) The method of claim 77, wherein said RNA/DNA hybrid is a product of subtraction.

82. (previously presented) The method of claim 77, wherein said RNA/DNA hybrid is the product of a method comprising the steps of normalization and subtraction in any order or of a method comprising a single normalization/subtraction step.

83. (currently amended) A method of isolating single-stranded cDNA comprising the steps of treating a hybrid comprising RNA nonspecifically bound to cDNA and having single-stranded RNA sites with an enzyme that degrades single-stranded RNA to degrade single-

stranded RNA, removing the degraded single-stranded RNA, and recovering the cDNA.

84. (currently amended) A method of preparing normalized and/or subtracted cDNA comprising the steps of adding an enzyme that degrades single-stranded RNA driver nonspecifically bound to cDNA tester and having single-stranded RNA sites, and removing the degraded single-stranded RNA driver.

85. (previously presented) The method of claim 77, wherein said DNA or cDNA is full-length or full-coding length cDNA.

86. (previously presented) The method of claim 1 employed to prepare one, two, or more libraries.

87. (canceled).

88. (canceled).

89. (previously presented) The method of claim 1, in which subtraction is performed and normalization is performed to a R_0T value of from 5 to 10.

90. (previously presented) The method of claim 13, wherein the chemical tagging is performed on ice.